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Effect of different sample volumes on the DNA extraction of *Aspergillus fumigatus* from whole blood

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Abstract

Five methods were compared, using conventional PCR, for the isolation of DNA from *Aspergillus fumigatus* conidia from 1–3-mL samples of whole blood. A lower detection threshold of *Aspergillus* conidia was achieved using 3-mL rather than 1-mL samples with three of five methods tested.

Keywords: *Aspergillus fumigatus*, DNA extraction, PCR (polymerase chain reaction), sample volume, whole blood

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Invasive fungal infections are increasing in immunocompromised individuals and *Aspergillus fumigatus* is one of the most important fungal pathogens that endanger this group of patients. The detection of fungal DNA in blood or other clinical material via PCR is highly sensitive and has been frequently studied [1–5]. The initial problem is the extraction of fungal DNA from clinical specimens. Despite many efforts to find an appropriate and sensitive method, there is no agreement about an optimal procedure for the extraction of *A. fumigatus* DNA from whole blood [6–9]. In addition, to date, no study investigating the effect of different sample volumes on the efficiency of DNA extraction from blood samples has been carried out.

In the present study, we have analysed this effect on the extraction of *A. fumigatus* DNA using five different methods with two different sample volumes.

Methods include different techniques, such as a phenol–chloroform extraction and three commercially available kits: the Invisorb Spin Plant Mini kit (Invitek, Berlin, Germany), the FastDNA kit (MP Biomedicals, Heidelberg, Germany) and the High Pure PCR template preparation kit (Roche, Mannheim, Germany), with the latter kit using with two different pre-treatment steps.

Suspensions of *A. fumigatus* (DSM 790) conidia were prepared from fresh *A. fumigatus* colonies cultured on Sabouraud dextrose agar plates (Becton & Dickinson, Heidelberg, Germany) as described previously [10]. Dilutions were prepared by diluting the original suspension serially 1:10 with sterile saline and checked by plating on Sabouraud dextrose agar. For sample preparation, whole blood was drawn from a healthy volunteer and spiked with *A. fumigatus* conidia yielding final concentrations of 1×10^5 to 1 CFU/mL, respectively. The blood samples were divided into 5×1 mL and 5×3 mL, respectively. Hypotonic lysis of erythrocytes and enzymatic

lysis of leukocytes was performed as reported previously [6]. Samples processed by three methods were treated with lyticase: DNA extraction using phenol–chloroform, Invisorb Spin Plant Mini kit and High Pure PCR template preparation kit. Samples were centrifuged, the pellet resuspended in 50 mM NaOH and incubated at 95°C for 10 min. After centrifugation, the pellet was resuspended in 500 μL of lyticase solution (50 mM Tris (pH 7.6), 10 mM EDTA, 28 mM mercaptoethanol, 0.3 mg/L recombinant lyticase (MP Biomedicals)) and incubated for 30 min at 37°C. After an additional centrifugation step, the Invisorb kit and the High Pure kit were used in accordance with the manufacturer's instructions.

In addition, the High Pure kit was evaluated in combination with glass bead-beating. The EWCLB-treated sample was transferred into a new tube containing approximately 60–70 mg of glass beads (acid-washed, 710–1180 μm; Sigma, Steinheim, Germany).

The suspension was vortexed for 10 s and transferred into a new tube. After centrifugation, the supernatant was discarded and the sample treated according to the manufacturer's instructions.

For extraction using the FastDNA kit, samples were centrifuged after WCLB-incubation and 1.2 mL of the supernatant transferred into FastDNA tubes containing Lysing Matrix A (garnet matrix and one ¼ inch ceramic sphere). Cells were disrupted using the Fast Prep FP120 cell disrupter (Qbiogene, Illkirch, France) at 5.5 m/s for 30 s. Treatment was repeated twice and samples processed according to manufacturer's instructions.

Samples extracted with phenol–chloroform were treated as described previously [11].

Negative controls were included in each extraction method to detect contaminations extracted DNA was amplified by PCR using a primer set for the fungal 18S rRNA multicopy gene which does not cross-hybridize with human DNA [1]. The primer sequences were: 5′-ATTggAggg CAAgTCTggTg and 5′-CCgATCCCTAgTCggCATAg. PCR was performed in a volume of 55 μL, including 4 μL of nucleotides (Roche), 2 μL of 25 mM $MgCl_2$, 5 × PCR buffer, 1 μL of each primer (20 μM; TIB Molbiol, Berlin, Germany), 0.25 μL of Taq polymerase (1 U/μL; Applied Biosystems, Branchburg, NJ, USA) and 7 μL of template DNA.

PCR conditions were set as described previously [1]. PCR products were visualized on a 1.2% agarose gel. The results were confirmed in an independent experiment using PCR–ELISA (data not shown).

The five different extraction methods yielded rather different results revealing the importance of the technique chosen for extraction (Table 1). All commercially available kits used for this study achieved a higher sensitivity than the conventional method with phenol–chloroform. Additionally, the processing time was shorter (up to 7 h) if a kit was used. The phenol–chloroform extraction showed the lowest sensitivity for detection of *Aspergillus* DNA independent of volume size, followed by the Invisorb kit. The sample volume was crucial for all methods tested except the High Pure kit when used with glass bead-beating. This method showed the same detection limit for both volumes, each recovering

TABLE 1. Summary of detection cut-offs of *Aspergillus fumigatus* conidia in whole blood extracted using the indicated methods, and their duration

Extraction method	Costs/sample (€)	Duration (h)	Detection cut-off (CFU/mL)	
			1 mL	3 mL
Phenol–chloroform	0.50	10	10^4	10^4
Invisorb Spin Plant Mini kit	3.00	3	10^4	10^3
FastDNA kit	3.20	3	10^3	10^1
High Pure kit/lyticase	2.70	5	10^3	10^1
High Pure kit/glass beads	2.20	4	1	1

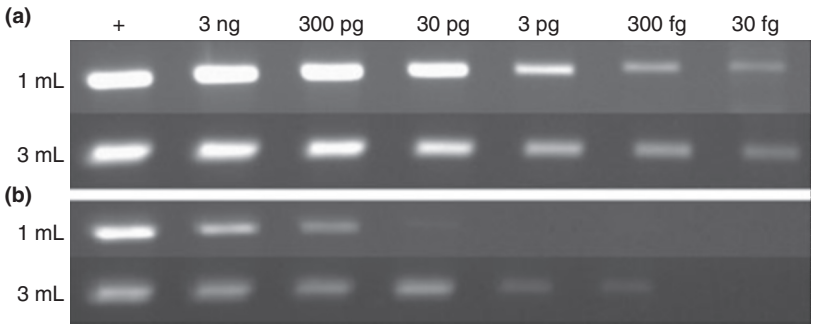

FIG. 1. Gel electrophoresis of samples extracted by High Pure PCR template preparation kit. Sample volumes of 3 and 1 mL were compared in combination with (a) cell wall disruption using glass beads and (b) treatment with lyticase. **+**, Positive control.

approximately 30 fg of DNA, which corresponds to 1 CFU/mL [12]. All other methods proved to be more sensitive with sample sizes of 3 mL rather than 1 mL. The FastDNA kit and the High Pure kit in combination with lyticase allowed detectection of approximately 300 fg of DNA if a volume of 3 mL whole blood was used. By contrast, a sample volume of 1 mL achieved a detection cut-off of 3 pg, which comprises a difference of 2 log units showing a 100-fold decline in sensitivity (Fig. 1).

These results showed that an initial sample volume of 3 mL yielded a higher sensitivity compared to a 1-mL sample volume. Hence, 3 mL should be the preferred volume. In addition, bead-beating was more efficient in disrupting the fungal cell walls than lyticase (Fig. 1) and a sample volume of 3 mL may be reduced to 1 mL with similar sensitivity in the case of the High Pure kit with bead-beating [7,13,14]. Other potential advantages of the treatment with glass beads are the saving of time and costs compared to the enzymatic lysis (Table 1). However, in terms of clinical relevance, the results should be interpreted with caution. Clinical specimens might behave differently than spiked blood obtained from a healthy volunteer. Additionally, there is a lack of clarity regarding the ideal specimen type. Whole blood was used in the present study because it is easy to obtain and the risk of contamination is relatively low in contrast to material such as bronchoalveolar lavage fluid. Nevertheless, further studies should be performed using bronchoalveolar lavage fluid, serum or plasma to confirm the existing findings.

Transparency Declaration

The authors declare that they have no conflicts of interest in relation to this work.

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